45). Therefore, FRA in normal adult tissues is inaccessible through the bloodstream and hence normal tissues are spared from side effects of drugs that target FRA.

[0012] While in normal tissues FRA is not accessible via the vasculature, several malignant tumors over-express FRA, including those of the ovaries, lungs, colorectal, renal, and, recently, breast carcinomas (5, 6, 44, 45). In these tissues, FRA is accessible through the bloodstream. Thus, the restricted expression of FRA on the apical surface of normal tissue and its overexpression on malignant cells, makes it an attractive therapeutic target for cancer treatment.

[0013] Moreover, two studies recently found FRA expression to be strongly associated with ER/PR-negative and TNBC (>80%) status as well as poor prognosis with FRA expression associated with metastatic breast cancer and worse overall and disease-free survival (6,7). Taken together, these findings make FRA an attractive target for treating TNBC particularly.

[0014] The current disclosure includes use of folate itself (folic acid) as well as folate analogs that are also capable of binding to folate receptors.

[0015] Illustrative embodiments of folate analogs include folinic acid, pteropolyglutamic acid, and folate receptor-binding pteridines such as tetrahydropterins, dihydrofolates, tetrahydrofolates, and their deaza and dideaza analogs. The terms "deaza" and "dideaza" analogs refer to the art-recognized analogs having a carbon atom substituted for one or two nitrogen atoms in the naturally occurring folic acid structure, or analogs or derivatives thereof. For example, the deaza analogs include the 1-deaza, 3-deaza, 5-deaza, 8-deaza, and 10-deaza analogs of folate. The dideaza analogs include, for example, 1,5-dideaza, 5,10-dideaza, 8,10-dideaza, and 5,8-dideaza analogs of folate. Other folate analogs useful as complex forming ligands include the folate receptor-binding analogs aminopterin, amethopterin (methotrexate), N^{10}-methylfolate, 2-deamino-hydroxyfolate, deaza analogs such as 1-deazamethopterin or 3-deazamethopterin, and 3',5'-dichloro-4-amino-4-deoxy-N^{10}-methylpteroylglutamic acid (dichloromethotrexate). The foregoing folic acid analogs are conventionally termed folates, reflecting their ability to bind with folate-receptors. Additional analogs of folic acid that bind to folic acid receptors are described in U.S. Patent Application Publication Nos. 2005/0227985 and 2004/0242582.

[0016] The basic antibody structural unit includes a tetramer. Each tetramer is composed of two pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region (the fragment crystallizable or "Fc" region) primarily responsible for effector function. Depending on the amino acid sequence of the heavy chain constant region, a given human antibody or immunoglobulin can be assigned to one of five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM. Several of these classes may be further divided into subclasses (isotypes), e.g., IgG1 (gamma 1), IgG2 (gamma 2), IgG3 (gamma 3), and IgG4 (gamma 4), and IgA1 and IgA2. The structures and three-dimensional configurations of different classes of immunoglobulins are well-known and relevant sequences are available in publicly-accessible databases, such as PubMed. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)).

[0017] "Antibody-dependent cell-mediated cytotoxicity" (ADCC) refers to a cell-mediated reaction in which non-specific cytotoxic cells that express Ig Fc receptors (FcRs) (e.g. Natural Killer (NK) cells, monocytes, neutrophils, and macrophages) recognize bound antibody (or a fragment thereof) on a target cell and subsequently cause lysis of the target cell (e.g., a cancer cell). FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a conjugate disclosed herein in vitro ADCC assays, such as those described herein and/or in U.S. Pat. No. 5,500,362, or 5,821,337 can be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC).

[0018] Monoclonal antibodies currently in use in the clinic for the treatment of a number of diseases, including several malignancies, are of the IgG isoform. These antibodies were engineered with the sole intent to inhibit a major signaling pathway critical for the progression of disease, such as Herceptin to inhibit cell growth, in the treatment of HER2+ breast cancer. Later, it was discovered that some of these antibodies, in addition to their primary function, were also capable of stimulating ADCC through the binding of their Fc portion to their respective FcγIII receptors on macrophages, PMNs and NK cells (21,22). This finding led to the modification of some of these antibodies and the development of combined therapies using FcγIII-activating immunocytokines (G-CSF) in conjunction with bispecific antibodies targeting both cancer cells and FcγIII to increase their ADCC potential (23,25). However, the ADCC response mediated through FcγIII is weak possibility due to their low expression level, the presence of high levels of competing IgG antibodies in serum, their presence on other non-cytotoxic cells and the presence of inhibitory FcγII receptors (21,26) and thus, requires administration of various immunocytokines such as G-CSF to increase their effectiveness.

[0019] FcαR1, on the other hand, is expressed only on cytotoxic cells and in high numbers on PMNs making this receptor a very potent mediator of ADCC independent of any immunocytokine co-factors (27). The concept of targeting FcαR1 using monoclonal or bispecific IgA antibodies targeting this receptor to elicit cancer cell killing by PMNs has been tested successfully in vitro (10, 11, 28-33) where IgA also showed superiority over IgG. However, research into the potential therapeutic use of FcαR1 is hampered by the lack of adequate mouse models because mice do not express FcαR1 and the lack of established models for production and purification of high levels of IgA (34).

[0020] The current disclosure demonstrates the binding of novel FRA-binding conjugates to FRA-expressing cancer cells, such as TNBC cells. Upon binding, the conjugate is able to recruit and activate neutrophils (PMNs), the major white blood cell in humans, to destroy the cancer cell. These findings suggest the use of a conjugate of this type could effectively kill cancer cells and thus represents a promising effective targeted therapeutic treatment for cancer, such as TNBC.

[0021] Accordingly, the present disclosure provides novel FRA-binding conjugates (in one embodiment, referred to as